LIFE: International Journal of Health and Life-Sciences
ISSN 2454-5872

Eldeen et al., 2015

Volume 1 Issue 1, pp. 24-37

Year of Publication: July, 2015

DOI- https://dx.doi.org/10.20319/lijhls.2015.11.2437

This paper can be cited as: Eldeen, I., Napiah, N. E. M., & Mohamad, H., (2015). Efficacy of Three Mangrove Plants against 5-Lipoxygenase, Acetylcholinesterase Enzymes and Five Pathogenic Bacterial Strains. LIFE: International Journal of Health and Life-Sciences, 1(1), 24-37.

EFFICACY OF THREE MANGROVE PLANTS AGAINST 5-LIPOXYGENASE, ACETYLCHOLINESTERASE ENZYMES AND FIVE PATHOGENIC BACTERIAL STRAINS

Ibrahim MS. Eldeen
Institute of Marine Biotechnology, University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
Eldeen24@umt.edu.my

Nor Emera. M. Napiah
Institute of Marine Biotechnology, University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

Habsah Mohamad
Institute of Marine Biotechnology, University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

Abstract

Mangroves are highly productive ecosystem with various important economic and environmental functions. They are the key elements in marine environment that produce diverse metabolites to adapt with the requirement of their challenging ecosystem. This makes them an interesting source for natural bioactive molecules.

In this study, we investigated inhibitory effects of extracts from Avicenna lanata, Ceriops tagal and Sonneratia alba against 5-lipoxygenase, acetylcholinesterase enzymes and four pathogenic bacterial strains using in vitro models.

Best dual inhibitory effects against the two enzymes was recorded for the methanolic and ethylacetate bark extracts (final concentration used in the assay was 90 µg/ml) of Sonneratia alba and dichlro-root extarct of C.tagal (inhibition percentage ranging between 74-91%). Roots of Ceriops tagal showed the highest activity against lipoxygenase (93%), but was slightly weaker against AchE (83%).

Antimicrobial properties of the extracts was determined using the microdilution assay. A. lananta (bark) showed the best antimicrobial efects with the lowest minimum inhibitory concentration (MIC) value of 90 µg/ml against S.aureus, E.coli and K. pneumoniae. Methanolic root and leaf extracts of C.tagal showed the same MIC values against S.aureus. Phytochemical analysis indicated the presence of alkaloids, steroids and tannins in the investigated plant parts. These results support the ethnobotanical uses of these plants. Chemical profiling, isolation and determination of mechanism of actions of the observed bioactivities are currently in progress in our laboratory.

Keywords

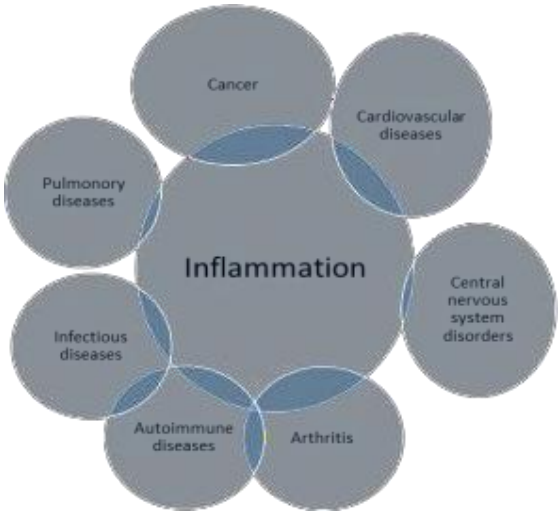
Mnagroves, Natural Products, Inflammation Lipoxyygenase

1. Introduction

Mangroves are highly productive ecosystem associates with diverse economic and environmental functions. The term is used to designate halophytic and salt resistant marine tidal forests comprising of trees, shrubs, palms, epiphytes, ground ferns and grasses (Premanathan et. al., 1999). Apart from the ecological roles, ethnobotanical evidences indicated the utilization of a number of mangrove plant species in traditional medicine against human, animal and plant pathogens. Due to their special growth environment, mangroves produce diverse group of metabolic substances with wide range of biological activities including inflammatory related ailments and infectious diseases. The medicinal properties of mangrove trees therefore, provide a wide domain for biological applications that worth further investigation (Eldeen and Effendy, 2013)

(Simone et.al. 2005) Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules. The inhibitions of numerous rate-limiting processes could be important in the successful treatment of an inflammatory

disorder (Premanathan et. al., 1999). Prolong inflammtion may lead to pathological conditions including immune-mediated diseases such as multiple sclerosis, acute neurodegeneration following ischemia or trauma, and, more recently, chronic neurodegenerative diseases (Simone et.al., 2005).



The non-steroidal anti-inflammatory drugs (NSAIDs) are proven to be effective for treatment of inflammation symptoms due in most cases to their ability to inhibit prostaglandin synthesis by binding reversibly and irreversibly to the enzyme. However, their draw back or toxicities are due to their ability to block synthesis of the housekeeping prostaglandin as a result of inhibition of COX-1 (Portonova et.al., 1996). Leukotriene modifiers are a class of drugs used for the treatment of inflammatory related disorders such as asthma. These drugs have introduced recently into clinical practices (Samaria, 2004). Their mechanism of actions are believed to be due to their effects on leukotrienes by interrupting the 5- lipoxygenase pathway. leukotrienes are pro-inflammatory mediators that could rapidly increase the inflammatory responses. They may also contribute to development of certain types of tumor such as colon tumor (Samaria, 2004; chan et.al. 2012). Development of compounds that inhibit 5-LOX or both COX-2 & Lipox would be advantageous due to their ability to target both proteins, enhancing their individual anti-inflammatory effects and reducing their associated side effects (chan et.al., 2012 ; Rao et.al., 2012;). For this target, natural products containing chemical entities with a wide structural

diversity serve as a useful source of potential compounds with possible dual LOX /COX inhibitors.

Among the other endogenous mechanisms that regulate the inflammatory response are the cross-action between the immune and nervous systems (Rao et.al. 2012). An In vivo study indicated that electric stimulation of the vagus nerve attenuates the inflammation during endotoxemia in rats, and that acetylcholine (ACh), the main parasympathetic neurotransmitter, effectively deactivates peripheral macrophages and inhibits the release of pro inflammatory mediators. Inhibition of acetylcholinesterase enzyme therefore, is also relevant with its possible actions on some of the pro inflammatory mediators (Rao et.al. 2012) .

This reports aimed to evaluate inhibitory effects of 5-liopoxygenase, acetylcholinesterase enzymes, and eradication of microbial growth by three mangrove plants Avicennia lanata, Ceriops tagal and Sonneratia alba.

2. Material and Methods

2.1 Plant Material and Extraction

Plant materials including leaves, root and/or bark of the three mangroves were collected from Setiu Wetland, Terengganu. A voucher specimen (Eldeen 9,10,11) was deposited in the Herbarium of The Institute of Marine Biotechnology, University Malaysia Terengganu.

A.lanata *C.tagal* *S. alba*

The collected materials were, dried in an oven at 55◦C for 7 days, powdered and extracted sequentially using dichloromethane, ethyl acetate and methanol. Residue obtained were concentrated to dryness and kept in room temperature for further bioassay tests.

- **Phytochemical Screening**

The plant extarcts were subjected to phytochemical screening test to detrmine the presence of, carbohydrates, phenols tannins, saponins, glycosides, steroids, terpenoids and alkaloids.

- **Bioactivity Screening**

Lipoxygenase inhibitor screening assay

The 5- lipoxygenase (5-Lipox) inhibitory effects of the plant extracts were evaluated using the Lipox inhibitor screening assay kit (Item No. 760700; Cayman Chemical, USA). The assay was performed based on the supplier's provided protocol. In this assay, the detection reaction was confirmed to be senstitive to hydroperoxides at various positions within the fatty acid of any carbon length, therefore, the reaction is seen to be suitable as a general detection method for Lipox , and can be used for screening of natural products from different origins with unknown mechanism of actions. Inhibition percentages were calculated by subtracting the avergae absorbance of the 100% initial activity from the abosrbance of inhibitors.

- **Acetylcholinesterase Enzyme Inhibitory Activity**

Inhibition of acetylcholinesterase biosynthesis by the plant extracts was investigated using the micro- plate assays based on Ellman's method with modifications as described before (Eldeen et.al., 2005). The enzyme activity was measured by observing the increase of a yellow color produced from thiocholine when it reacts with the dithiobis-nitrobenzoate ion. The bioassay was carried out using the 96-well microplate. One 96-well microplate was used for three samples. 25µl of 15mM ATCI in water, 125µl of 3mM DTNB in buffer C, 50µl of buffer B, 25µl of sample, methanol and galanthamine, and 25µl of AchE was loaded into each well of the 96-well microplate. 25µl of each sample (resuspended to a concentration of 100 µg/ml using methanol to give final concentration of 10 µg/ml in the assay) was added the first row (row A) and two fold serially diluted. The galanthamine was used as the positive control while methanol as the negative control in the test. The absorbance was measured at 405 nm immediately after the addition of the enzyme. The rate of reaction was calculated. Three replicate of the test was done.

- **Micro-Dilution Antibacterial Assay**

To determine the minimum inhibitory concentration (MIC) of the extracts, 96-well microplates was used following the serial dilution technique methods (Eloff, 1998). Five bacterial strains–two Gram-positive: Bacillus cereus and Staphylococcus aureus, and three Gram-negative: Escherichia coli, Salmonella typhimurium and Kleibsiella pneumoniae was subcultured overnight in the Mueller Hinton broth. On the next day, 100 µl of each subcultured bacteria was inoculated into the new vials containing 10 ml of new MH broth. The plant extracts were re-disolved in sterile water to a concntration of 5 mg/ml (final concentration of 1.25 mg /ml

in the assay) was used. The assay was performed as described before (Eldeen et.al., 2010 ; Eldeen, 2014).

To indicate the bacterial growth, 50µl of Resazurine blue (1 mg/5ml millipore water) was added into each of the well. Bacterial growth in the wells was indicated by a purple/pink color, whereas clear blue wells indicated inhibition by the tested substances (Eldeen, 2014).

3. Results and Discussion

3.1 Phytochemical Constituents

The phytochemical tests indicated the presene of alkaloids, carbohydrates, glycosides, saponins, steroids, tannins and terpenoids in the leaf and bark of S.alba. With the exception of A. Lanata, tannins and terpenes were also detected in almost all the plant part tested. Glycoside and steroids were not detected in the leaf and root of C.tagal and leaf of A.lanata (Table 1).

Phenols, tannins, glycosides, steroids, terpenoids, alkaloids and flavonoids are secondary metabolites exist in higher plants. These secondary metabolites are needed for the plants to interact with their environment for the protection against biotic or abiotic stresses such as infections, wounding, UV irradiation, exposure to ozone, pollutants, and herbivores (Oksman-caldentey and Barz, 2002) .Some of these classes are parent molecules for biosynthesis of numerous structurally and functionally diverse plant-derived end product moleules which play essential roles in plant physiology (Oksman-caldentey & Barz, 2002 ; Korkina et.al., 2011 ;).

The spectrum of secondary metabolites produced by plants increase with the environment and harsh growth conditions. Due to the harsh condition of mangrove environment, such metabolic agents are expected to be diverse in qauntity and quality including their wide range of biological activities (Oksman-caldentey and Barz, 2002). Plant-derived phenylpropanoids represent the largest group of secondary metabolites produced by higher plants. Their medicinal roles as antioxidants, anti-inflammatory, wound healing, and antibacterial agents were previously highlighted. However, these molecules also reported to possess limiting factors indicated by their potential toxicity and difficulties with sensitization (Oksman-caldentey and Barz, 2002).Therefore, biological evaluation and assessment of their efficacy is urgently needed.

Table 1: *Phytochemical Screening of Extracts of the Three Mangrove Plants A. Lanata, C.tagal and S.alba*

Plant name	Plant part analyzed	alk	car	gly	sap	Ster	tann	terp
Avicennia lanata	leaf	+	+	-	-	-	-	-
	bark	-	+	+	-	+	+	-
	root	-	+	+	+	+	+	-
Ceriops tagal	leaf	+	+	-	+	-	+	+
	bark	nt	nt	nt	nt	Nt	nt	nt
	root	+	+	-	+	-	+	+
Sonneratia alba	leaf	+	+	+	+	+	+	+
	bark	+	+	+	+	+	+	+
	root	nt	nt	nt	nt	nt	nt	nt

Al=alkaloids; car= carbohydrates; gly= glycosides; sap= saponins; ster= Steroids; tann= tannins; terp= terpenoids. nt= not tested.

- **5- Lipoxygenase Enzyme Inhibitory Activity**

Results of 5- lipoxygenase enzyme inhibitory effects by the three mangrove plant extracts are given in Table 2.Extracts showed inhibitory effects with percentage of inhibition >80% are considered highly active when tested at final concentration of 90 µg/ml in the assay. Both leaf and root extracts of C.tagal showed strong activities against the enzyme (exception was ethyl acetate and methanolic root extracts).

Table 2: *Lipoxygenase and Acetylcholinesterase Enzymes Inhibitory Effects by Plant Extracts (10 mg/ml) of Three Mangrove Plants: A.lananta, C.tagal and S.alba when Tested using in Vitro Models*

Plant species	Plant part analyzed	5-Lipox inhibition (%)		AchE Inhibition (%)	
		Extracts tested		Extracts tested	
		Dichloromethane Ethyl acetate	Methanol	Dichlorometha Ethyl acetate	Methanol

Avicennia lanata	Leaf	66±3.1	69±1.9	55±3.1	61±2.9	61±2.9	67±2.7
	Bark	71±3.0	70±1.7	64±5.5	63±3.1	66±4.6	57±1.3
	Root	67±2.8	65±2.6	58±5.2	55±4.6	71±3.8	73±5.5
Ceriops tagal	Leaf	80±0.8	83±3.9	93±2.4	72.4±1.1	54.3±1.4	43.7±1.8
	Root	91±4.0	58±3.0	30±2.5	83±2.3	40.63±2.4	38.73±2.7
Sonneratia alba	Leaf	77±3.4	78±3.3	81±2.4	68.7±4.4	62.4±1.4	68.2±4.0
	Bark	81.2±5.9	74.6±3.7	85±3.1	76.2±3.2	74.6±3.4	81.3±2.9

Inhibition obtained (%) is expressed as mean ± S.D. Percentage Inhibition of prostaglandin synthesis by indomethacin (standard) was 80±1.9% for COX-1 and 69±2.4% for COX-2. Inhibition (%) of acetylcholinestersae enzyme by galanthamine (20µM) was 93±3.2%

All the extracts from S.alba also showed strong to moderate activities against the enzyme. Percentage of inhibition ranging between 85 and 74%.

It is well known that one of the traditional method to reduce inflammation is through inhibition of enzymes associated with the arachidonic acids including lipoxygenase and cyclooxygenase. Epidemiological and clinical studies on the other hands suggest that COX-2 inhibitors have a better GI toxicity profile than indiscriminative NSAID. However, others also raised concerns regarding effects of of leukotrienes in GI toxicity (Gale et.al. 2007). Therefore, it is relevant to look for both COX and 5- Lipox inhibitors which may provide better anti-inflammatory effects (Gale et.al. 2007). This was also supported by data from clinical trials which confirmed that, interrupting the leukotriene pathway offers a new opportunity for treating inflammatory related ailments such as asthma (Samaria, 2004). The inhibitory effects observed by both C.tagal and S.alba therefore are in line with this concept of dual inhibitory actions as these extracts also possessed inhibitory effects against cyclooxygenase (data not published yet). The potential anti-inflammatory properties of C.tagal is in agreement with previously reported biological activities of some mangrove plants (Simlai and Roy, 2013) . Indomethacin was used as positive control. It was previously reported to possess dual inhibitory effects against COX and 5-Lipox [17].

- **Acetylcholinesterase Enzyme Inhibitory Activity**

The cholinesterase inhibitory activity of the plant extracts obtained by using the microplate assay are presented in Table 2. Different extracts of the three plants showed activities against acetylcholinesterase enzyme. Best inhibition percentage (83%) was obtained by dichloromethane root extract of C.tagal followed by, all bark extract of S.alba (74-81%), methanolic root extract of A.lanata (73%), dichloromomethane leaf of C.tagal (72%) and ethyl acetate root of A.lanata (71%).

Cholinesterase inhibitors increase the amount of acetylcholine at the neuronal synaptic cleft by inhibiting the enzyme responsible for the hydrolysis of acetylcholine and consequently improve neuronal transmission (Zangara, 2003; Eldeen et.al. 2008 ;). Inhibition of acetylcholinesterase activity may indicate potential for therapeutic use in treatment of cognitive disorders. On the other hand, these biological effects could be evaluated together with the potential anti-inflammatory effects possessed by the plant as indicated by the inhibition of 5-lipox. Since inhibition of AchE also contributed or accounted for anti-inflammatory effects (Rao et.al. 2012), we have correlate the activities against the two enzymes (Fig 1). C.tagal (root) and S.alb (bark) appeared to be the best in terms of dual inhibitory effects. This is also in line with the concept that some of anti-inflammatory drugs may lead to a protective effect reducing the incidence of eurodegenerative disorders (McGeer et.al. 1996; Howes and Houghton, 2003 ;). Galanthamine was used as the positive control. It is an alkaloid with mechanism of actions believed to be on both peripheral and central nervous system (Heinrich and Lee, 2004). Both the active parts of the investigated plant confirmed the presence of alkaloids which may suggest similar mechanism of action with the Galanthamine.

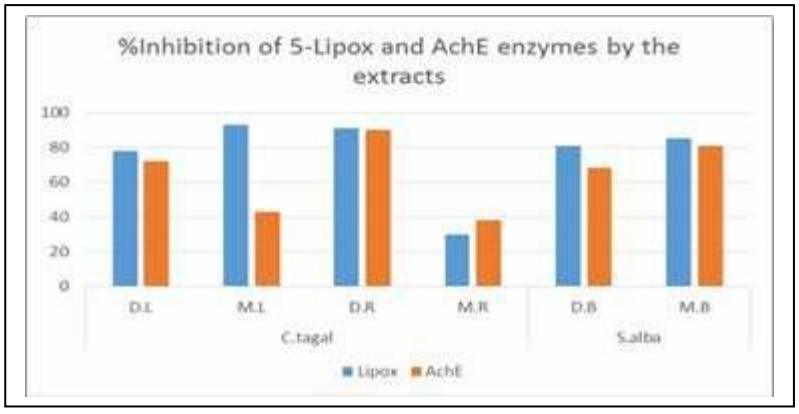


Figure 1: *D=Dichloromethane; M=Methanol; L= Leaf; B=Bark; R=Root*

- **Antimicrobial Activities of the Plant Extracts Obtained by Microdilution Assay**

For the antimicrobial properties of the investigated plant extracts, 66% of all the extracts tested against the pathogenic bacterial strains showed MIC values ≤ 0.97 mg/ml (Table 3). The leaf and bark extracts of S.alba showed activities against all the tested strains with MIC values ranging between 0.97-0.19 mg/ml. Methanolic bark and root extracts of A.lanata showed the lowest MIC values (90 µg/ml) against E.coli, S.aureus and K.pneumoniae. Similar effects also observed with the methanolic leaf and bark extracts of C.tagal against S.aureus.

Insert Table 3
(Refer to Annexure)

We reported previously antimicrobial properties of some mangroves species and eendophytes (Eldeen, 2014). The activities observed in this study by S.alba are in line with previous findings on the same or closely related species (Saad et.al. 2012). In a previous comparative antimicrobial activities studies on different extracts of aerial part of of Ceriops decandra, the methanol extract possessed the best activity (Vadlapudi and Naidu, 2009). This could be comparable to our current finding on the closely related species C.tagal using different bioassay test.

4. Conclusion

In this study different extracts from three mangrove plants: A.lanata, C.tagal and S.alba were evaluated for inhibitory effects against 5-Lipox and AchE enzymes beside antimicrobial properties. Phytochemical screening was also carried out to determine the major class of constituents. Methanolic bark and dichloro root extracts of S.alba and C.tagal respectively were found to possess the best dual inhibitory effects against both the enzyme tested (Fig1). The biological activities observed by leaf extracts in this study are interesting as it can lead to substitution of leaves for roots and bark during utilization of the plants. Harvesting of leaves for medicinal purposes is more sustainable compared to other plant parts such as roots and stem bark.

To our knowledge, this report is the first to highlight the determination of inhibitory effects of these mangrove plants against 5-liopx and AchE enzymes using these bioassay models.

Our efforts are now focused on determination of mechanism of actions of these molecules using cell lines based bioassays.

5. Acknowledgment

This work was funded by the Fundamental Research Grants (FRGS), managed by Research and Innovation Affairs University Malaysia Terengganu, and arranged by The Research Management Centre (RMC).

References

Chan, K. Y., Mohamad, K., Ooi, A. J., Imiyabir, Z., & Chung, L. Y. (2012). Bioactivity-guided fractionation of the lipoxygenase and cyclooxygenase inhibiting constituents from Chisocheton polyandrus Merr. Fitoterapia, 83(5), 961-967.

De Simone, R., Ajmone-Cat, M. A., Carnevale, D., & Minghetti, L. (2005). Activation of α7 nicotinic acetylcholine receptor by nicotine selectively up-regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures.Journal of neuroinflammation, 2(1), 4..

Eldeen, I. M. (2014). Isolation of 12 Bacterial endophytes from some mangrove plants and determination of, antimicrobial properties of the isolates and the plant extracts. International Journal of Phytomedicine, 6(3), 425-432. Oksman-Caldentey, K. M., & Barz, W. H. (Eds.). (2002). Plant biotechnology and transgenic plants (Vol. 92). CRC press.

Eldeen, I. M. S., Elgorashi, E. E., & Van Staden, J. (2005). Antibacterial, anti-inflammatory, anti- cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. Journal of Ethnopharmacology, 102(3), 457-464.

Eldeen, I. M., & Effendy, M. A. (2013). Antimicrobial agents from mangrove plants and their ndophytes. Microbial pathogens and Strategies for combating them: Science, Technology and Education. FORMATEX Microbiology Book Series. Formatex Research Centre: Badajoz, Spain, 872-882.

Eldeen, I., Van Staden, J., & Elgorashi, E. (2010). Ethnopharmacology of some African trees. VDM Verlag Dr. Müller Aktiengesellschaft & Co. KG Dudweiler Landstr, 99, 66123.

Eldeen,Van Heerden., & Van Staden. (2008).Termilignan b and arjuric acid from the roots of Terminalia sericea and their biological activities.Planta Med, 74(411-413).

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta medica,64(8), 711-713.

Gale, G. A., Kirtikara, K., Pittayakhajonwut, P., Sivichai, S., Thebtaranonth, Y., Thongpanchang, C., & Vichai, V. (2007). In search of cyclooxygenase inhibitors, anti-Mycobacterium tuberculosis and anti- malarial drugs from Thai flora and microbes. Pharmacology & therapeutics, 115(3), 307-351.

Heinrich, M., & Teoh, H. L. (2004). Galanthamine from snowdrop—the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. Journal of ethnopharmacology, 92(2), 147-162.

Howes, M. J. R., & Houghton, P. J. (2003). Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function.Pharmacology Biochemistry and Behavior, 75(3), 513-527.

Korkina, L., Kostyuk, V., De Luca, C., & Pastore, S. (2011). Plant phenylpropanoids as emerging anti-inflammatory agents. Mini reviews in medicinal chemistry, 11(10), 823-835.

Mantri, P., & Witiak, D. T. (1994). Inhibitors of cyclooxygenase and 5-lipoxygenase. Current medicinal chemistry, 1(4), 328.

McGeer, P. L., Schulzer, M., & McGeer, E. G. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease A review of 17 epidemiologic studies. Neurology, 47(2), 425-432.

Portanova, J. P., Zhang, Y., Anderson, G. D., Hauser, S. D., Masferrer, J. L., Seibert, K., ... & Isakson, P. C. (1996). Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. The Journal of experimental medicine, 184(3), 883-891.

Premanathan, M., Arakaki, R., Izumi, H., Kathiresan, K., Nakano, M., Yamamoto, N., & Nakashima, H. (1999). Antiviral properties of a mangrove plant, Rhizophora apiculata Blume, against human immunodeficiency virus.Antiviral Research, 44(2), 113-122.

Rao, C. V., Janakiram, N. B., & Mohammed, A. (2012). Lipoxygenase and cyclooxygenase pathways and colorectal cancer prevention. Current colorectal cancer reports, 8(4), 316-324.

Saad, S., Taher, M., Susanti, D., Qaralleh, H., & Awang, A. F. I. B. (2012). In vitro antimicrobial activity of mangrove plant Sonneratia alba. Asian Pacific journal of tropical biomedicine, 2(6), 427-429.

Samaria, J. K. (2000). Anti-leukotriene drugs: are they really useful. J Indian Acad Clin Med, 5(1), 41-4.

Simlai, A., & Roy, A. (2013). Biological activities and chemical constituents of some mangrove species from Sundarban estuary: An overview. Pharmacognosy reviews, 7(14), 170.

Vadlapudi, V., & Naidu, K. C. (2009). In vitro antimicrobial activity Ceriops decandra against selected aquatic, human and phytopathogens. Int. J. Chem. Tech. Res, 4, 1236-1238.

Yamawaki, I., Suzuki, M., & Ogawa, K. (1994). Piperazinealkanol ester derivatives of indomethacin as dual inhibitors of 5-lipoxygenase and cyclooxygenase. Chemical & pharmaceutical bulletin, 42(4), 963- 971.

Zangara, A. (2003). The psychopharmacology of huperzine A: an alkaloid with cognitive enhancing and neuroprotective properties of interest in the treatment of Alzheimer's disease. Pharmacology Biochemistry and Behavior, 75(3), 675-686.

Table 3: *Antimicrobial Properties of Extracts Obtained from the Mangrove Plants A.lanata, C.taga. and S.alba as determined by the Micro-Dilution Assay. Results are Minimum Inhibitory Concentrations (MIC) Values in µg/ml*

Plant species	Plant part analysed	Dichloromethane					Ethylacetate					Methanol				
		Bacteria tested					Bacteria tested					Bacteria tested				
		Bc	Sa	Ec	Kp	S.t	Bc	Sa	Ec	Kp	S.t	Bc	Sa	Ec	Kp	S.t
Avicennia lanata	Leaf	312	320	320	na	na	na	220	550	90	260	na	na	na	na	na
	Bark	na	220	na	320	na	na	320	na	320	na	na	90	90	90	na
	Root	na	na	650	na	520	na	na	550	na	na	na	90	90	320	na
Ceriops tagal	Leaf	na	450	580	520	na	na	160	290	220	290	260	90	190	130	an
	Root	na	320	580	420	na	na	160	290	260	na0.29	260 0.26	940.1	160	130	na 0.260
Sonneratia alba	Leaf	870	580	510	580	na	410	430	210	310	970	680	210	430	390	680
	Bark	730	430	610	580	530	580	780	510	530	na	210	210	190	210	680
Gentamicin sulphate	Bc=	Bc=0.488±0.41; Sa=1.587±2.18; Ec=0.208±0.26; Kp=0.392±0.55; St=1.563±0.00														

Bacteria: Bc = *Bacillus cereus;* Ec = *Escherichia coli;* Kp = *Klebsiella pneumoniae;* Sa = *Staphylococcus aureus; St. Salmonella typhimurium.* N a = not active at the highest concentration uses (1.25 mg/ml).